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Cold-active enzymes and their applications in industrial fields - A review

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Abstract **– Cold-active enzymes are produced by organisms adapted to permanently cold habitats. Due to the depressive effect of low temperatures on reaction rates, these enzymes have to be adapted to secure appropriate reaction rates in those organisms that often thrive in environments characterized by temperatures close or below the freezing point of water. They are encountered in all prokaryotic or eukaryotic organisms adapted to cold such as microorganisms, invertebrates, insects and fish originating from the Arctic and Antarctic zones, as well as from alpine regions, glaciers or permafrost zones. They are characterized by a high specific activity at low temperatures, in any case higher than that of their mesophilic and thermophilic counterparts. This higher specific activity is generally accompanied by a decrease in thermal stability illustrated by a shift of the apparent optimum towards low temperatures, and by an important decrease in the thermodynamic stability characterized by a significantly lower stabilization enthalpy. The generally low stability induces an increase in the flexibility of the overall edifice or of crucial zones for activity of the molecular structure. There is apparently a continuum in the adaptation since some enzymes display extreme adaptation illustrated by a severe shift of the activity towards low temperatures whereas others are moderately adapted. This probably depends on their position in a metabolic pathway, on their intracellular or extracellular localization, on the environmental temperature and on the evolutionary history of the organisms.**

Keywords **– Cold-Active Enzymes, Metagenomics, Psychrophile, Microbial Enzymes.**

I. INTRODUCTION

Over 70% of the ecosphere is classified as cold environments, covering oceans, soils, glaciers, lakes and sea ice (Priscu & Christner, 2004). Antarctica, the southernmost continent, is also the coldest with 90% of the continent covered in ice sheets (Fretwell *et al*., 2013). Sea ice specifically, covers almost 15% of the area, making it one of the largest biomes in this region (Thomas and Diekmann 2002). Sea ice is a semisolid matrix, containing a network of brine channels wherein sea ice microorganisms can be found.

Despite the unfavorable conditions for life due to low temperature, high solar radiation, low nutrient availability, and extreme aridity, Antarctica has been successfully colonized by microorganisms. Microbes are one of the most important elements in nutrient recycling and organic matter mineralization in this extreme environment owing to their expressing cold-adapted enzymes (Gerday *et al*. 2000). Despite having a slow growth rate, several psychrophiles, e.g., *Psychrobacter cryohalolentis*, have been reported to have high metabolic rates as physiological response towards cold temperature to counteract the low biochemical rates at low temperature (Amato and Christner 2009). Since a high level of ATP is required to maintain a high metabolic rate, psychrophiles produce cold-adapted enzymes that work efficiently at low temperature to utilize any nutrient sources available in their environment.

Microorganisms from low-temperature environments possess numerous adaptations to the extreme conditions

including synthesis of cold-active enzymes with shifted temperature optimum, reduced thermal stability, and high affinity to their substrates (Cavicchioli *et al*. 2002; D'Amico *et al*. 2002; Maayer *et al*. 2014). Due to their unique proper-ties, such enzymes can find applications in pharmaceutical and food industries, bioremediation, fine chemical synthesis, etc. (Elleuche *et al*. 2014; Littlechild 2015; Margesin *et al*. 2002).

This review is to summarize the potential of cold-active enzymes originating from organisms that live in permanently cold environments, and methods that can used to get of this enzymes, and their applications in difference industries.

II. Source of Cold-Active Enzymes

Microorganisms thriving at low temperatures are known for a long time Psychrophilic organisms (Farrell & Rose, 1967; Morita, 1966). The name "psychrophile" is of Greek origin and the words "Psychros" mean cold and "Philos" mean loving (i.e. cold- loving). They have been called also "cryophiles" and "rhigophiles". Both of these words were derive d also from Greek and also had the essential meaning as psychrophiles that was used first by Schmidt (1902). Psychrophilic organisms have been classified in two groups (Stokes, 1963): (1.) Obligate psychrophiles are having optimal growth temperature of 20 °C. (2.) Facultative psychrophiles are having optimal growth temperature of >20 °C.

Cold-adapted microorganisms are broadly categorized into psychrophiles as they are cold-loving microorganisms and they show optimal growth at less than 15 °C and psychrotrophs as they are cold-tolerant microorganisms and they show optimal growth at 20 – 25 °C. (Helmke and Weyland 2004; Cowan *et al.* 2007). The notable features of cold- adapted microorganisms are their survival at low temperatures, due to increased unsaturated fatty acid content in the membrane and increased solute concentrations inside the cell s (D'Amico *et al.* 2006; De Maayer *et al.* 2014). Microorganisms and other higher organisms inhabit places like cold water, ice, frozen soil, glaciers, etc. Microorganisms inhabiting at these low temperatures function well and are often found in combination with other extreme environmental conditions, for example, high salinity, low pH, high pH and high pressure. Mykytczuk *et al*. (2013) reported the bacteria *Planococcus halocryophilus* Or1 that were isolated from high Arctic permafrost showed virtuous growth and repro-duce at 15 °C. It has been reported that metabolically active bacteria inhabiting at extreme low temperatures as low as 32 °C (Bakermans and Skidmore 2011) show possibility of life even further. Both categories of extremophilic microorganisms like psychrophilic (cold-loving) or psychrotolerant (cold-adapted) are found residing at low temperatures of the earth, which include high mountain s, glaciers, ocean depths, polar areas, shallow subterranean and refrigeration appliances. They are also found on the surfaces of plants and animal s living in c old environments, where temperatures never exceed 5 °C. The mesophilic yeasts grow between 5 °C and 35 °C. An extensive variety of col d-loving microorganisms have been found inhabiting these low-temperature environments as some of them be long to gr am-negative bacteria, e.g. *Pseudoalteromonas* sp., *Moraxella* sp., *Psychrobacter* sp., *Polaromonas* sp., *Psychroflexus* sp., *Polaribacter* sp., *Moritella* sp., *Vibrio* sp. and *Pseudomonas* sp.; some belong to gram-positive bacteria, e. g. *Arthrobacter* sp., *Bacillus* sp. and *Micrococcus* sp.; some archaea also belong to psychrophiles, e. g. *Methanogenium* sp., *Methanococcoides* sp. and *Halorubrum* sp.; yeasts like *Candida* sp. and *Cryptococcus* sp.; and fungi like *Penicillium* sp. and *Cladosporium* sp. have also been isolated from cold environments (Cavicchioli *et al*. 2002; Deming 2002; Margesinetal. 2002; Feller and Gerday 2003; Georlette *et al*. 20 04). Permanently cold environments such a s the polar region, marine environment and deep water are the

p laces where psychrophiles or cold-loving microorganisms are mostly found (Sabri, Melara, & Algom, 2001). Environments where psychrophilic and psychrotrophic microorganisms are inhabiting are supposed to work on the biodegradation of organic matter and the cycling of essential nutrients (Lambo and P. 2006; Welander 2005; Ruberto *et al.* 2005). It has been reported that col d-adapted microorganisms provide an extensive biotechnological prospective over heuseof organisms and their enzymes which operate at higher temperatures (Georlette *et al*. 2004; Margesin*et al*. 2002). There are quite a lot of potential applications of cold-adapted enzymes in the food processing industries (Margesin and Schinner 1994). Psychrophilic microorganisms are required to swell the important applications of cold-active enzymes in different areas of food processing.

III. Isolation of Cold-Active Enzymes

Historically, two conventional methods have been widely used to isolate new enzymes from environ-mental samples. The first technique uses enrichment culturing followed by screening, or direct plating of environmental samples for screening, of bacteria using selective media. The second method involves the construction of metagenomic libraries and activity- or sequence-based screening. In addition, a combination of these methods (enrichment culturing, metagenomic library construction, and functional screening) has been tested for the ability to detect novel genes encoding alcohol oxidoreductases in environmental samples (Knietsch *et al*. 2003).

3.1. *Enrichment Technique*

Enrichment culture is basically an isolation technique designed to make conditions of growth very favorable for an organism of interest while having an unfavorable environment for any competition. It is the use of certain growth media to favor the growth of a particular microorganism over others, enriching a sample for the microorganism of interest. This enrichment is generally done by introducing nutrients or environmental conditions that only allow the growth of an organism of interest. Enrichment culture techniques are used to increase a small number of the desired organisms to detectable levels. This allows for the detection and identification of microorganisms with a variety of nutritional needs (Liu *et al.,* 2016).

Enrichment culturing is often employed for isolating microbial species of interest from soil and marine habitats. The enrichment culturing includes two methods depending on type of media utilized: One is submerged (liquid medium), which is easy to control and optimize environmental and nutritional parameters, and the other is solid-state fermentation in which solid materials (raw or processed) are used as substrates (Mahitha and Madhuri, 2016). Enrichment-dependent novel enzyme production in liquid fermentation method commonly involves the utilization of physical parameters like temperature, pH, incubation time, static and shaking conditions, and/or chemical properties like carbon and nitrogen sources and substrate and inoculum concentration as determining factors for the enzyme-producing isolates, thus providing the chance to grow and proliferate only the adapting species to the determining physical and/or chemical factor (Shah and Patel, 2014). If the case is solid media utilization, certain factors have no influence as they are not maintained like static and stirring of the fermentation medium, and some new factors come into play as the substrate type (raw or processed).

3.2. *Metagenomics*

Although the uncultured microorganisms cannot be studied using traditional microbiological and biochemical methods, their DNA can be extracted directly from environmental samples (metagenomic DNA) and cloned for

sequencing or expression in Escherichia coli or other surrogate hosts (Handelsman *et al*. 1998).

Metagenomics has emerged as a strategic approach to study microbial communities, without culturing individual organisms, through the analysis of their DNA using both bioinformatics and experimental methods such as sequence analysis, DNA hybridization, gene expression, proteomics, metabolomics, and enzyme activity assays (Handelsman 2004; Lorenz *et al*. 2002; Uchiyamaand Miyazaki 2009; Vieites *et al*. 2009). The metagenomic studies of soil and marine communities have already revealed many new species and proteins including bacteriorhodopsin and numerous unknown protein families (Beja *et al*. 2000; Gilbert and Dupo nt 2011; Ty son *et al*. 2004; Venter *et al*. 2004; Yoosephetal. 2007). The vast sequence diversity in environmental metagenomes suggests a similar magnitude of metabolic and biochemical diversity (Dinsdale *et al*. 2008; Yooseph *et al*. 2007), but the latter is impossible to comprehensively describe based only on sequence analysis due to the presence of the large number of unknown or poorly characterized genes. This necessitates the development of experimental approaches for metagenome research including new cultivation technologies, meta-transcriptomics, meta-proteomics and activity based screening methods (Ferrer *et al*. 2007; Giovannoni and Stingl 2007; Ram *et al*. 2005; Simon and Daniel 2011; Uchiyama and Miyazaki 2009). Agar plate-based screening of metagenomic gene libraries for enzymatic activity represents a simple and direct approach to identify metagenomic enzymes. This approach has been applied to screen different met a genomic gene libraries for esterase, lipase, cellulose, protease, lactase and other activities (Ferrer *et al*. 2009; Lo-renz and Eck 2005; Steeleetal. 2009). Screening of metagenomic gene libraries and purified proteins has greatly expanded the scope of enzyme applications and the number of enzymes useful for bio catalysis. These studies have identified more than 130 new nitrilases and many other enzymes including celluloses, lipases, carboxyl esterases and lactases (Beloqui *et al*. 2006; Chowetal. 2012; Ferrer *et al*. 2005a; Hess *et al*. 2011; Kourist *et al*. 2007; Robertson *et al*. 2004; Schmeisser *et al*. 2007).

For research and commercial applications, which require considerable amounts of active recombinant protein, the enzymes of interest were produced using a heterologous overexpression system using Escherichia coli as a host. This host was because it has numerous advantages, including inexpensive culture conditions, rapid growth, and easy manipulation (Rosano and Ceccarelli 2009). However, the conventional approaches to discovering new enzymes, and the later steps of heterologous expression for downstream applications, have several intrinsic problems. First, the source bacterium for enzymes is often unavailable in the metagenomic library. Second, the detection rate is extremely low due to the difficulty of functionally expressing the enzyme genes in heterologous E. coli. Finally, the recombinant proteins, although overexpressed, frequently form inactive insoluble aggregates (Heath *et al*. 2009).

IV. Importance of Cold-Active Microbial Enzymes

Cold-adapted enzymes exhibit higher catalytic activities at low temperature compared to mesophilic enzymes (Siddiqui *et al*. 2013), owing to their structural flexibility and modifications at the active site for easier substrate binding. Studies on cold-adapted enzymes have highlighted several unique characteristics compared to their mesophilic and thermophilic homologs. These include reduced core hydrophobicity, increased surface hydrophobicity, fewer electro-static interactions, fewer disulfide bridges, higher glycine residue content, increased formation of loop structures, and fewer proline residues (Feller and Gerday 2003; Gerday *et al*. 2000 ; Siddiqui *et al*. 2013).

Discoveries of new cold-active enzymes have gained a lot of attention due to their huge potential in various industrial applications. Since these enzymes exhibit high catalytic efficiency at low to moderate temperatures compared to their mesophilic and thermophilic counterparts, they can provide substantial economic benefit as their application results in the saving of energy (Cavic-chioli *et al*. 2011). As only a small amount of the enzyme is needed for efficient catalysis at low temperatures, cold-adapted enzymes are being increasingly applied in industrial processes, particularly in the food, beverage, and textile industries (Marx *et al*. 2007).

Furthermore, cold-active enzymes are usually thermo labile and can be inactivated after a reaction by mild heat input. This is especially important in sequential reactions where there is a need to inactivate an enzyme after it has performed its function, while maintaining the conditions that allow the other enzymes involved in the reaction to function (Cavicchioli *et al*. 2011). Esterases (EC3.1.1.1) grouped under the α/β hydrolase fold family are one of the largest groups of enzymes that have been studied. Esterases hydrolyze soluble esters with short acyl chain (less than 10 carbons), unlike lipases that prefer long chain triglycerides. Structural elucidation of esterases has revealed a catalytic triad of Cys–His–Asp in their active site. As they belong to the α/β hydrolase fold family, their structure consists of central hydrophobic β-sheets surrounded by amphiphilic α-helices at the periphery (Nardini and Dijkstra 1999).

Dienelactone hydrolase (EC 3.1.1.45), an enzyme under the α/β hydrolase family, shares the same structural features as esterases. It is involved in the 2, 4-dichlorophenoxyacetic acid (2, 4-D) degradation path-way where it converts chlorodienelactone to maleylacetate in cells (Kumar *et al*. 2014), and is one of the key enzymes involved in the degradation of chloroaromatic compounds via ortho and meta chlorocatechol cleavage pathways after which the product is catabolized into Krebs cycle intermediates (Moiseeva *et al*. 2002; Schlömann 1994). Dienelactone hydrolases have been classified into three groups, trans, cis- and both trans and cis, which is based on their substrate specificity. Studies on the catalytic mechanism of dienelactone hydrolase from *Pseudomonas knackmussii* showed that the catalytic triad is composed of Cys–His–Asp, with a G–x–C–x–G–G motif sequence around the catalytic cysteine (Pathak and Ollis 1990).

The psychrophilic yeast *Glaciozyma antarctica* used in this study was isolated from sea ice near the Casey Research Station, Antarctica and can grow in the temperature range 4–20 °C (Boo *et al*. 2013). The yeast is able to produce antifreeze proteins and several cold-adapted enzymes like chitinase and protease that are active at low temperature which allows it to thrive in extreme environments (Alias *et al*. 2014; Hashim *et al*. 2013; Ramli *et al*. 2011).

Another advantage of cold active enzymes especially in terms of food industry is prevention of bacterial contamination and undesirable chemical side reactions which can take place at high temperatures (Kuddus and Ramteke 2012; De Gobba *et al*. 2014; Siddiqui 2015). Due to their superior features, cold active enzymes have potential for industrial applications and they have attracted considerable attention in recent years.

4.1. *Amylases*

Amylases are among the most important enzymes due to their wide spectrum of biotechnological applications in many industrial processes such as starch liquefaction, textile, food, baking. In general, amylases can be divided into two categories, exo-amylases and endo-amylases, based on their distinct modes of catalysis. Among endo-amylases, α-amylase (EC.3.2.1.1) is one of the most important industrial endo-amylases, which is capable

of hydrolyzing the internal α-1, 4 glycosides linkages to glucose, maltose, and dextrin, while retaining the α-anomeric configuration in the products (Gupta *et al*. 2003). Classified according to the similarity of the primary structure of the well-defined amino acid sequence of the catalytic domains, α-amylase mainly belongs to the GH13 family, together with the GH57, GH119, and eventually GH126 families (Fickoblean *et al*. 2011). As a ubiquitous enzyme, α-amylase is produced by many species, including animals, plants, and microorganisms. Of these, α-amylases from microorganisms are preferred because of their plasticity for genetic manipulation and potential for economical bulk production (Neerja *et al*. 2013). Among the different types of α-amylases, cold-active α-amylase is superior to its mesophilic and thermophilic homolog in most industrial applications due to its high activity and high stability under the room temperature or below. Recently, it was found that marine microorganisms in cold environment (e.g., deep sea and polar regions) are the potential sources of cold-active α-amylases (Feller *et al*. 1992; Zhang and Zeng 2008; Lu *et al*. 2010; Kuddus *et al*. 2012b; Qin *et al*. 2014; Kim *et al*. 2017). Furthermore, some cold-active α-amylases have been purified from the above marine microorganisms including *A. haloplanctis* (Feller *et al*. 1992), *Nocardiopsis* sp. 7326 (Zhang and Zeng 2008), Pseudoalteromonas sp.GS230 (Lu *et al*. 2010), *Zunongwangia profunda* (Qin *et al*. 2014) and *A. agilis* (Kim *et al*. 2017). The optimum temperatures for activity of these cold-active α-amylases were 29–35 °C.

In general, the reported cold-active α-amylases and their microorganism sources are still limited. Moreover, the cold-active α-amylases may be required at the operating tempera-ture below 29 °C in some industrial applications (e.g., food processing). Therefore, it is important to search for novel cold-active α-amylases with desirable properties for diverse industrial applications from as many different sources as possible.

4.2. *β-D-Galactosidases*

The main sources of cold-active β-D-galactosidases are psychrophilic and psychrotolerant microorganisms and the vast majority of these enzymes have been isolated from *Arthrobacter* species. The reason for this apparent preference can be explained by the fact that most of these strains were isolated from soil samples and *Arthrobacter* sp. are basic soil bacteria. As far as we know, only two cold-active β - D -galactosidases have been identified, isolated and biochemically characterized to date from eukaryotic microorganism, the yeast-like fungus *Guehomyces pullulans* (Song *et al*. 2010; Nakagawa *et al*. 2006). Most of the known bacterial strains producing cold-active β-D-galactosidases were isolated from low temperature environments. For example, *Arthrobacter* sp. C2-2 was isolated from fell field soil (Karasová-Lipovová *et al*. 2003), *Planococcus* sp. SOS Orange from a hyper saline pond in Antarctica (Sheridan and Brenchley 2000) and *Carnobacterium piscicola* BA was isolated from a soil sample taken in late winter from a field treated with whey in Pennsylvania, USA (Coombs and Brenchley 1999). Several other interesting examples of bacterial strains producing cold-active β-D-galactosidases include *Pseudoalteromonas* sp. 22b, which was isolated from the digestive tract of Antarctic krill, *Thysanoessa macrura* (Cieśliński *et al*. 2005) and *Alkalilactibacillus ikkense*, which was isolated from the Ikka columns in South-West Greenland (Schmidt and Stougaard 2010). However, the living conditions of these microbes are generally impossible to recreate in the laboratory (using conventional methods) and culturable microorganisms represent less than 1 % of those occurring in nature (White *et al*. 2000). For this reason, Wang *et al*. investigated the direct cloning of the DNA found in environmental samples collected from an oil field in the Heilongjiang Province, China and constructed a series of metagenomic libraries. Using this method, the researchers successfully identified the ZD410 gene, which encoded a cold-active β-D-galactosidase (Wang *et al*.

2010).

Besides the origin of cold-active β-D-galactosidases, an even more important criterion for the comparison of their enzymatic properties is their affiliation to the glycoside hydrolase (GH) families. One hundred and thirty-three GH families have recently been distinguished, and proteins belonging to the same family have been reported to have an amino acid sequence homology of no less than 30 % (Poltorak *et al*. 2007). It is currently commonly accepted, that the GH1, GH2, GH35 and GH42 families contain enzymes that possess β-D-galactosidase activity. In terms of their ability to affect the removal of lactose from dairy products, enzymes belonging to the GH2 family perform more effectively than enzymes belonging to any of the other families because they demonstrate higher activity towards lactose. It is note-worthy, however, that β-D-galactosidases from the GH42 family possess a variety of other activities that are not exhibited by β-D-galactosidases in the GH2 family. Several proteins belonging to the GH42 family have also been reported to exhibit β-D-fucosidase (EC 3.2.1.38) and α-L -arabinosidase (EC 3.2.1.55) activities (Di Lauro *et al*. 2008; Lee *et al*. 2011; Kosugi *et al*. 2002; Saishin *et al*. 2010; Sheridan and Brenchley 2000). Most of the cold-active β-D-galactosidases that have been identified, isolated and biochemically characterized to date are members of the GH2 and GH42 families, although a few cold-active enzymes with β-D-galactosidase activity have also been found belonging to the GH35 family. It has been also reported that some "cold- loving" microorganisms possess more genes that are capable of encoding enzymes with β-D-galactosidase activity. For example, the psychrotolerant bacterium *Arthrobacter* sp. B7 carries genes of three different is enzymes with β-D-galactosidase activity, which can be classified into the GH2, GH35 and GH42 families (Trimbur *et al*. 1994 ; Gutshall *et al*. 1995, 1997). It has also been found that the bacterium *Carnobacterium piscicola* BA produces two β-D-galactosidases, which are members of the GH35 and GH42 families, respectively (Coombs and Brenchley 1999, 2001). Furthermore, *Arthrobacter* sp. ON14 has been reported to contain two cold-active β-D-galactosidase s, which are members of the GH2 and GH42 families (Xu *et al*. 2011). Coombs and Brenchley (2001) proposed that the presence of two or more GH enzymes in one microorganism could be explained by the need for the synergistic activity of these enzymes to assist in the degradation of other polysaccharides with α - and β-galactosidase linkages, which are abundant in the environment. This hypothesis was based on the fact that the genes of two cold-active β-D-galactosidases and one α–galactosidase are located on one operon in *Carnobacterium piscicola* BA. By cooperating in the degradation of certain sugars, these enzymes could provide good alternative sources of carbon for their microorganisms (Coombs and Brenchley 2001).

4.3. *Xylanases*

Xylan is one of the major structural polysaccharides in plant cells and the second most abundant polysaccharide after cellulose in nature. Given its structural complexity, enzymatic hydrolysis of xylan needs cooperative action of a variety of enzymes (Moreira and Filho 2016). Among these xylanolytic enzymes, endo-xylanase (EC 3.2.1.8), which is normally termed xylanase, is particularly important because it catalyzes cleavage of the internal β-1, 4-glycosidic linkages of xylan backbone, thereby generating xylo-oligosaccharides (XOS) with low degree of polymerization (DP) (Moreira and Filho 2016). On the basis of amino acid sequence and three-dimensional structure, majority of endo-xylanases are categorized as glycosyl hydrolase family 10 and 11 (GH10 and GH11) (http: //www.cazy.org/). To date, a large number of endo-xylanases have been characterized and applied in various fields of industry, such as pulp bio bleaching (Walia *et al*. 2017), waste

paper deinking (Dhiman *et al*. 2014), animal feed production (Harris and Ramalingam 2010), bread making (Butt *et al*. 2008), biofuel production (Bhalla *et al*. 2015), and prebiotic production (Jain *et al*. 2015).

Generally, an enzyme performance is highly sensitive to reaction temperature. Biotechnological application of xylanase (similar to many other enzymes) is normally hindered by its temperature-dependent catalytic property. Therefore, in recent years, the use of extremophilic xylanases to satisfy the industrial demands for xylanase that can catalyze under harsh conditions has been attracting interest. Currently, a large number of thermophilic xylanases have been exploited (Kumar *et al*. 2018). By contrast, less attention is given to psychrophilic or cold-active xylanases, despite their considerable potential applications in many industrial processes. Cold-active xylanase can be especially used in industrial processes where undesirable chemical reactions occur at high temperature (Santiago *et al*. 2016), and they are particularly suitable in food and feed industry applications (Cavicchioli *et al*. 2002, 2011). An example of cold-active xylanase application is its usage for improving dough stability and flexibility and for increasing bread volume and crumb structure (Collins *et al*. 2006; Butt *et al*. 2008; Dornez *et al*. 2011).

In addition, cold-active xylanase possesses the general advantage of a cold-active enzyme for application, such as energy-saving characteristic and inherently broad substrate specificity relative to its thermophilic counterparts (Santiago *et al*. 2016). To date, the number of cold-active xylanases is largely limited (Santiago *et al*. 2016). No more than 20 cold-active xylanolytic enzymes have been heterogeneously expressed and characterized (Santiago *et al*. 2016).

These cold-active xylanases were generally obtained from cultured psychrophilic microorganisms or metagenomes (Vester *et al*. 2015). Mining of enzyme from unexplored genome databases (such as GenBank) can be an alternative method to characterize novel enzymes (Lauro *et al*. 2010; Gong *et al*. 2013). Therefore, we provided special attention to sequenced genomes of marine microorganisms that are considered reservoirs of novel and extremophilic enzymes (Littlechild 2015). *Luteimonas abyssi*, XH031T, a novel species of *Luteimonas*, was isolated from deep-sea sediment of the South Paciic Gyre (genome accession number in GenBank: NZ_KQ759763) (Fan *et al*. 2014). According to genome annotation, a xylanase (GenBank accession numbers: WP_082672697.1, termed as laXynA) is presumably produced by the strain.

4.4. *Lipolytic Enzymes*

Lipolytic enzymes are classified into eight families (Arpigny and Jaeger 1999; Hausmann and Jaeger 2010) with a large group of carboxyl esterases (EC 3.1.1.1) which hydrolyze various carboxylic esters and produce the corresponding alcohols and organic acids as products (Arpigny and Jaeger 1999; Bornscheuer 2002; Satoh and Hosokawa 2006). Most known esterases are α/β-hydrolases with the Ser-His-Asp catalytic triad, and these enzymes are widely distributed in microorganisms, plants and animals. Recently, the erythromycin e sterase family of α/β-hydrolases has been identified and is proposed to use a His-Glu pair for the catalysis (Morar *et al*. 2012). Moreover, several active esterases have also been identified in the β -lactamase superfamily, which have a different structural fold and use the Ser residue of the β-lactamase sequence motif Ser-x-x-Lys as a catalytic nucleophile (Wagner *et al*. 2002). Many esterases have a broad substrate range suggesting that they have evolved to function in carbon utilization and catabolic pathways. Together with lipases (EC 3.1.1.3), esterases are the most frequently used hydrolases in bio catalysis (Bornscheuer and Kazlauskas 2006). In addition, these enzymes also show high regiospecificity and stereo specificity, which makes them useful biocatalysts in organic

synthesis, especially for the production of enantiopure secondary alcohols and for the resolution of primary alcohols and carboxylic acids (Baumann *et al*. 2000; Bornscheuer and Kazlauskas 2006; Bornscheuer *et al*. 2005). Many esterases are also stable and active in organic solvents, where they catalyze synthesis reactions including synthesis of enantiopure building blocks, flavours and fragrances (Bornscheuer and Kazlauskas 2006).

4.5. *Proteases*

Cold- active proteases have a wide range of industrial and technological applications. They are considered as key enzymes in food industry (in particular in low-temperature and low-moisture cheeses ripening as a micro biological 10 Cold-Active Enzymes from Cold-Adapted Yeasts 307 alternative to rennet), in seafood processing for removing both scales and skin from fish as well as for extracting carotenes from shellfish, and in meat processing to give tenderness to refrigerate d products without loss of flavors (Huston 2008).

A few cold -active proteases have been isolated from both psychrophilic and psychrotolerant yeasts, purified to homogeneity and characterized. They were often characterized by maximum activity in environments with relatively low pH (3.5-4 .5). The discovery of a cold-active aspartyl proteinases (molecular mass ¼ 36 kDa) from an Ant arctic strain of *Cryptococcus humicola* (now *Vanrija humicola*) dated back to the early 1990s. This enzyme was active at temperatures ranging from 0 to 45 °C, with an optimum at 37 °C, and retained about 15% of its maxim um activity at 0 °C (Ray *et al*. 1992).

The species *G. antarctica* was shown to be an active producer of cold–active proteases, in particular of a few subtilases belonging to the proteinase K subfamily (subtilizing family). The purified enzymes exhibited very high specific activity, a wide specificity, with the greatest affinity to substrates characteristic of chymotrypsin and chymotrypsin-like enzymes. Such high specific activity exhibited by *G. antarctica* subtilases is similar to that found in most other proteases produced by bacteria as well as by a few mesophilic yeasts and dimorphic yeast -like fungi (Tobe *et al*. 1976; Ogrydziak 1993; Białkowska and Turkiewicz 2014).

An extracellular serine proteinase was isolated from an Ant arctic strain of *L. antarcticum* (now *G. antarctica*), purified to homogeneity and characterized. The sequence of its 35 N-terminal amino acid residues exhibited 31% identity to that found in proteinase K (Pazgier *et al*. 2003). Likewise, Turkiewicz *et al*. (2003) characterized a glycosylated serine proteinase (molecular mass ¼ 34.4 kDa) produced by a strain of the same species: a quite low optimal temperature (25 °C), a poor thermal stability, and a high catalytic efficiency from 0 to 25 °C were found.

Alias *et al*. (2014) isolated the PI12 protease gene from genomic and mRNA sequences of *G. antarctica* and amplified it by rapid amplification of cDNA ends strategy. The recombinant PI12 protease (molecular mass ¼ 99 kDa) obtained using *Pichia pastoris* (now *Komagataella pastoris*) host at 20 °C exhibited over 40% identity with the subtilis in-like protease synthesized by *Rhodosporidium toruloides* (now *Rhodotorulat oruloides*), but no homo logy with other cold -active proteases.

On the contrary, Lario *et al*. (2015) isolated an extracellular protease from an Antarctic strain of *R. mucilaginosa*. The enzyme was purified to homogeneity and characterized. The purified protease presented optimal catalytic activity at pH 5 and 50 °C and high stability in the presence of high concentrations of NaCl. The ability of psychrophilic and psychrotolerant yeasts to produce cold–active proteases has been extensively reviewed (de (of) Garcı´a *et al*. 2012; Zaliha *et al*. 2012; Duarte *et al*. 2013; Białkowska and Turkiewicz 2014 ;

Chaud *et al*. 2016).

V. Applications of Cold-Active Enzymes

5.1. *Applications in Food Processing*

Consequently, cold-active enzymes have been applied in diverse industries involving food, detergents, biomass conversion, molecular biology, and baking (Feller and Gerday 2003).

It is not new but from long back microbial enzymes are used in foods and beverages. But incurrent food processing and beverage industries, the drift is to switch high temperature processes with low-temperature processes as it will help in maintaining the quality of the product. It has been found that low-temperature processing delivers commercial, economic and environmental benefits. The most fruitful advantages of low-temperature food processing are prevention of product s from food spoilage and contamination, energy saving s, the foodstuff will retain labile and volatile flavour compounds, quality of taste will be maintained and most importantly undesirable chemical react ions that may occur at higher temperatures will be minimized. Along these benefits, there will be higher control over cold- active enzymes as they can be inactivated at high temperatures (Pulicherla *et al*. 2011; Horikosh, 1999). Low-temperature processing will help in controlling the quality of material or foodstuff. The most focused application of enzymes in the baking industry is improvement of quality of bread including taste, and in the beverage industry, these potential enzymes are used in maintaining wine colour and clarity, and they are also used for reducing sulphur content. To boost the filterability and to develop or improve the flavour of final product s, different industrial enzyme s can be u sed. In regard to food and beverage enzymes, they make up the largest mark et for industrial enzyme applications. Some important microbial enzymes like α -amylases, peptide hydrolases, lipases, pectinase, lactase and catalases are added to foodstuff during food processing to imp rove specific characteristics of the food. Cold-active enzymes are also used for environmental bioremediation processes which include digesters, compo sting and oil degradation or xenobiotic biology applications; they are also applicable in molecular biology, biotransformation and heterologous gene expression in psychrophilic hosts to prevent formation of inclusion bodies (Feller *et al*. 1996).

5.2. *Applications in Biotechnological*

To date, many cold-adapted enzymes have been success-fully isolated, and expression and application studies have also been conducted (Zheng *et al*. 2011; Wang *et al*. 2016, 2007, 2011; Saba *et al*. 2012). Among these enzymes, cold-active proteases constitute, perhaps, the most important group, since their catalytic properties make them suitable for various biotechnological applications. For example, the use of cold-active proteases in the formulation of deter-gents would be of great advantage for cold washing. This would reduce the energy consumption as well as the wear and tear infected on textile fibers. The industrial dehairing of hides and skins at low temperatures using psychrophilic proteases or keratinases would not only save energy but also reduce the impact of toxic chemicals used in dehairing. Apart from these examples, cold-adapted proteases also have the potential for other interesting applications, such as bioremediation. In the previous 10 years, a great number of cold-adapted protease producing microorganisms have been isolated from different geographical regions, such as *Azospirillum* sp. from mountain soil (Oh *et al*. 1999), *Bacillus licheniformis* from glacier soil (Baghel *et al*. 2005), Clostridium sp. from the Antarctic (Alam *et al*. 2005), and *Colwellia* sp. from sea ice (Zhang *et al*. 2008),

etc. Moreover, a number of cold-adapted proteases have been successfully isolated and characterized in detail (Joshi and Satyanarayana 2013).

However, bacteria from deep-sea environments and their enzymes have received scant attention. The deep-sea regions are characterized by cold temperatures, high pressure, and complete darkness. The temperature in the deep zone is so low that it is just above freezing (between 0 and 3 °C) (Zhang *et al*. 2011). A number of very unusual organisms live in this part of the ocean, some of which do not require energy from the sun to survive, and instead use chemicals from deep inside the Earth. In addition, proteins and pep-tides constitute a substantial portion of the organic nutrients present in the deep-sea sediments as well as suspended particulate matter (Damare *et al*. 2006). Therefore, extracellular proteases may play a pivotal role in the physiology of deep-sea bacteria, and bacteria from deep-sea sediments could be a useful source of proteases. A possibility of obtaining a bacterium that produces cold-adapted proteases which may be exploited for effective cold washing and other industrial processes is certain. In light of this, we carried out a study on the isolation of cold-adapted extracellular proteases from bacteria isolated from deep-sea sediments of the Southern Indian Ocean. A total of 326 deep-sea isolates of bacteria from a depth of 5000 m in the South Indian Ocean were screened for protease enzymes. Many of these isolates grew and produced alkaline proteases at 5 and 20 °C under 1 bar pressure. *Planococcus* sp. M7 produced the highest amounts of protease enzymes and was selected for further studies.

5.3. *Applications in Biomedicine, Pharmaceuticals and Cosmetics*

Many pharmaceuticals, active pharmaceutical ingredients, fine chemicals, flavors, and fragrances are heat sensitive or/and volatile and hence must be synthesized at low temperatures at which cold -adapted enzymes are most active. In addition, it has been proposed that as a result of their proposed high structural flexibility, cold-adapted enzymes can operate at low water activity, such as in the aqueous/ organic and non-aqueous solvent systems frequently used during organic synthesis of complex molecules (Huston 2008; Karan *et al*. 2012). In this market sector, hydrolases, oxidoreductases, lyases, transferases, reductases, carboxylases, etc. are becoming more commonly used but only a few cold -adapted enzymes have been investigated and below an overview of these is given. The most widely used cold-adapted enzymes in this sector are lipases and esterases for the synthesis of optically pure intermediate com pounds of synthetic value. In fact, lipases (mainly CAL B) from *Candida antarctica* are among the most extensively and diversely used enzymes in organic synthesis. They are used in a broad range of surprisingly diverse application s, including the modification of sugars and sugar-r elated com pounds, desymmetrization of complex prochiral drug intermediates, and resolution of racemic alcohols and amines (Huston 2008 ; Kirk and Christensen 2002 ; Suen *et al*. 2004) during the synthesis of various pharmaceuticals (e.g., calcium antagonists as antihypertensive drugs , NK1/ NK2 antagonist for asthma treatment), cosmetics (e.g., iso-propylmyristate, a skin emollient), flavors, and fragrance esters. As discussed above (Sect. 19.4), cold-adapted ß-galactosidases are suited to the production of tagatose (an anti-hyperglycemic agent) and galacto-oligosaccharides (prebiotics). Moreover, these have also been shown to catalyze the synthesis of hetero oligosaccharides such as lactulose (for treatment of constipation and hepatic encephalopathy, use as a prebiotic, and use in diagnostics), galactosyl-xylose (use in diagnostics), and alkyl glycosides (foaming agent s) as well as glycosylated salicin (anti-inflammatory agent) from lactose (Pawlak-Szukalska *et al*. 2014). Also, as discussed previously, cold -adapted proteases can be used for the preparation of bioactive peptide s for use as antihypertensive, antioxidant, and immunoregulatory agents (Cazarin *et al*. 2015). Cold- adapted proteases are

currently being marketed as therapeutic agents against bacterial (biofilm breakdown) and viral (virus infectivity reduction) infections and in oral health care (plaque removal) and cosmetics (frown line reduction and dead or dried skin removal) (Fornbacke and Clarsund 2013). Other cold-adapted enzymes with potential in biomedical applications include a marine α-galactosidases which was shown to be capable of converting B red blood cells into the universal blood type O cells for use in transfusion therapy (Balabanova *et al*. 2010) and a cold-active nitroreductase as a cancer prod rug activating enzyme using low temperature therapy for activation (Çelik and Yetis¸ 2012).

5.4. *Other Applications*

In the textiles industry, cold -adapted amylases, cellulases, and lactases have been developed for the rapid desizing, or starch removal, of woven fabric s, bio-finishing of cellulosic fabrics, and less abrasive enzymatic stonewashing and bleaching of denim (Sarmiento *et al*. 2015). Cold- adapted enzymes, namely, cell wall degrading enzymes, amylases, lactases, lipase s, and phospholipases, have been suggested for improving the energy efficiency and costs of biofuel (bioethanol, biodiesel, and biogas) production processes. In particular, they should find application in cold-cook or no-cook processes, simultaneous saccharification and fermentation (Fester sen *et al*. 2005; Huston 2008; Gohel and Duan 2012; Ji *et al*. 2014; Wen *et al*. 2015), and low temperature biogas production (Akila and Chandra 2010).

Marine silicate in enzymes have been shown to be central in bio mineralization and in the synthesis of bio silicates found in marine diatoms, radiolarian, and sponges (Shimizu *et al*. 1998; Wang *et al*. 2012). This has important implications in materials science and indicates the potential of cold-adapted enzymes for the synthesis of a variety of nanostructured mineral/ organic composite materials under low temperature and mild chemical conditions. Examples of such materials include silica and siloxane polymers, bimetallic alloy nanoparticles, bimetallic perovskite-like materials, zirconia nanoparticles, spinel gallium oxide, etc.

VI. CONCLUSIONS

Psychrophilic organisms living in permanently cold habitats have developed numerous adaptations that allowed them to successfully thrive in low-temperature environments. Their enzymes are a key feature of this adaptation. They are much more active than their mesophilic counterparts at low and moderate temperatures and their high specific activity is undoubtedly due to an improvement of the flexibility of the active site or/and of other regions of the structure indirectly involved. This is induced by rather discrete structural modifications which can be located at long distance from the active site and which generally lead to a higher thermal instability of these enzymes, often associated with an even faster heat inactivation. If the general strategy adopted by nature consists in a weakening of the intramolecular forces that stabilize the structure of their mesophilic and a fortiori thermophilic counterparts, this strategy appears to be specific to each enzyme; depends on the position of the enzyme within a metabolic pathway; on its localization, intra-or extra-cellular; on its structural modification capability; on the environment of the organism and on its evolution history. As a consequence, there is a continuum in the adaptation to low temperatures; some cold-active enzymes indeed display high specific activity associated with low Tm while others show rather high Tm and stronger thermal dependence like their mesophilic counterparts.

It’s expected that more cold-active and cold- adapted microbial enzymes will be discovered in the near future,

due to their potentiality and characteristics that are highly needed in industries. Cold-active enzymes from psychrophilic microorganism can be most beneficial in food industries as they possess various advantages over the enzymes from other sources. The cold-active enzymes and their psychrophilic microbial sources cover a wide range of industrial applications. Cold-active enzymes from different sources are being used as additives in deter gents used for cold washing and as additives in food industries in fermentation, cheese manufacture, bakery and meat tenderizing. Cold-active enzymes from microbial origin are having interesting biotechnological as well as industrial applications. The few potential applications of these enzymes are mentioned here; some of them (e.g. β-galactosidase) are used for hydrolysis of lactose in milk, application of cellulases for bio-polishing and stone washing of textile products; pectinases are employed for extraction and clarification of fruit juices in food industry and for tenderization of meat and for improvement of taste in refrigerated meat using proteases. There is tremendous potential and use of microbial cold-active enzymes in different food industries. Due to their high stability at low temperatures, they are attracting more attention and are needed to be explored.

REFERENCE

[1] Alam SI, Dube S, Reddy GSN *et al* (2005) Purification and characterisation of extracellular protease produced by Clostridium sp. from Schirmacher oasis, Antarctica. Enzyme Microb Technol 36:824–831. https ://doi.org/10.1016/j.enzmi ctec.2005.01.011
[2] Alias N, Ahmad Mazian M, Salleh AB, Basri M, Rahman RN (2014) Molecular cloning and optimization for high level expression of cold-adapted serine protease from Antarctic yeast Glaciozyma antarctica PI12. Enzym Res 2014:1–20. 2014. (article ID 197938). https ://doi.org/10.1155/2014/19793 8
[3] Amato P, Christner BC (2009) Energy metabolism response to lowtemperature and frozen conditions in Psychrobacter cryohalolentis. Appl Environ Microbiol 75:711–718. https://doi.org/10.1128/ AEM.02193-08.
[4] Arpigny JL, Jaeger KE (1999) Bacterial lipolytic enzymes: classification and properties. Biochem J 343:177–183
[5] Baghel VS, Tripathi RD, Ramteke PW *et al* (2005) Psychrotrophic proteolytic bacteria from cold environment of Gangotri glacier, Western Himalaya, India. Enzyme Microb Technol 36:654–659. https ://doi.org/10.1016/j.enzmi ctec.2004.09.005
[6] Bakermans C, Skidmore ML (2011) Microbial metabolism in ice and brine at ˚5 degrees C. Environ Microbiol 13:2269–2278
[7] Balabanova LA, Bakunina IY, Nedashkovskaya OI, Makarenkova ID, Zaporozhets TS, Besednova NN, Zvyagintseva TN, Rasskazov VA (2010) Molecular characterization and therapeutic potential of a marine bacterium Pseudoalteromonas sp. KMM 701 α-galactosidase. Mar Biotechnol 12(1):111–120. doi:10.1007/s10126-009-9205-2
[8] Baumann M, Hauer B, Bornscheuer UT (2000) Rapid screening of hydrolases for enantioselective conversion of "difficult-to-resolve substrates". Tetrahedron Asymmetry 11:4781–4790
[9] Beja O, Aravind L, Koonin EV, Suzuki MT, Hadd A, Nguyen LP, Jovanovich SB, Gates CM, Feldman RA, Spudich JL, DeLong EF (2000) Bacterial rhodopsin: evidence for a new type of phototrophy in the sea. Science 289:1902–1906
[10] Beloqui A, Pita M, Polaina J, Martinez-Arias A, Golyshina OV, Zumarraga M, Yakimov MM, Garcia-Arellano H, Alcalde M, Fernandez VM, Elborough K, Andreu JM, Ballesteros A, Plou FJ, Timmis KN, Ferrer M, Golyshin PN (2006) Novel polyphenol oxidase mined from a metagenome expression library of bovine rumen: biochemical properties, structural analysis, and phylogenetic relationships. J Biol Chem 281:22933–22942
[11] Białkowska A, Turkiewicz M (2014) Miscellaneous cold-active yeast enzymes of industrial importance. In: Buzzini P, Margesin R (eds) Cold-adapted yeasts: biodiversity, adaptation strategies and biotechnological significance. Springer, Heidelberg, pp 377–396
[12] Boo SY, Wong CMVL, Rodrigues KF *et al* (2013) Thermal stress responses in antarctic yeast, Glaciozyma antarctica PI12, characterized by real-time quantitative PCR. Polar Biol 36:381–389. https ://doi.org/10.1007/s0030 0-012-1268-2
[13] Bornscheuer UT (2002) Microbial carboxyl esterases: classification, properties and application in biocatalysis. FEMS Microbiol Rev 26:73–81.
[14] Bornscheuer UT, Kazlauskas RJ (2006) Hydrolases in organic synthesis, 2nd edn. WILEY-VCH, Weinheim
[15] Bornscheuer UT, Ordonez G,Hidalgo A, Gollin A, Lyon J, Hitchman TS, Weiner DP (2005) Selectivity of lipases and esterases towards phenol esters. J Mol Catal B Enzym 36:8–13
[16] Butt MS, Tahir-Nadeem M, Ahmad Z, Sultan MT (2008) Xylanases and their applications in baking industry. Food Technol Biotechnol 46:22–31
[17] Çelik A, Yetis¸ G (2012) An unusually cold active nitroreductase for prodrug activations. Bioorg Med Chem 20(11):3540-3550. doi:10.1016/j.bmc.2012.04.004
[18] Cavicchioli R, Charlton T, Ertan H *et al* (2011) Biotechnological uses of enzymes from psychrophiles. Microb Biotechnol 4:449–460. https ://doi.org/10.1111/j.1751-7915.2011.00258 .x
[19] Cavicchioli R, Charlton T, Ertan H, Mohd Omar S, Siddiqui KS, Williams TJ (2011) Biotechnological uses of enzymes from psychrophiles. Microb Biotechnol 4:449–460
[20] Cavicchioli R, Siddiqui KS (2004) Cold adapted enzymes. In: Pandey A, Webb C, Soccol CR, Larroche C (eds) Enzyme technology. Asiatech Publishers, New Delhi, pp 615–638
[21] Cavicchioli R, Siddiqui KS, Andrews D, Sowers KR (2002) Low temperature extremophiles and their applications. Curr Opin Biotechnol 13:253–261
[22] Cazarin C, Lima G, da Silva J, Maro´stica M (2015) Enzymes in meat processing. In: Chandrasekaran M (ed) Enzymes in food and beverage processing. CRC, Boca Raton, pp 337–351. doi:10.1201/b19408-17
[23] Chaud LC, Lario LD, Bonugli-Santos RC, Sette LD, Pessoa Junior A, Felipe MD (2016) Improvement in extracellular protease production by the marine Antarctic yeast Rhodotorula mucilaginosa L7. N Biotechnol 33:807–814
[24] Chow J, Kovacic F, Dall Antonia Y, Krauss U, Fersini F, Schmeisser C, et al., (2012) The metagenomederived enzymes LipS and LipT increase the diversity of known lipases. PLoS One 7:e47665.
[25] Cieśliński H, Kur J, Białkowska A, Baran I, Makowski K, Turkiewicz M (2005) Cloning, expression, and purifi cation of a recombi-

-nant cold-adapted β-galactosidase from antarctic bacterium Pseudoalteromonas sp. 22B. Protein Expr Purif 39:27–34.
[26] Collins T, Hoyoux A, Dutron A, Georis J, Genot B, Dauvrin T, Arnaut F, Gerday C, Feller G (2006) Use of glycoside hydrolase family 8 xylanases in baking. J Cereal Sci 43:79–84.
[27] Collins T, Meuwis MA, Stals I, Claeyssens M, Feller G, Gerday C (2002) A novel family 8 xylanase, functional and physicochemical characterization. J Biol Chem 277:35133–35139.
[28] Coombs JM, Brenchley JE (1999) Biochemical and phylogenetic analyses of cold-active β-galactosidase from the lactic acid bacterium Carnobacterium piscicola BA. Appl Environ Microbiol 65:5443–5450.
[29] Coombs JM, Brenchley JE (2001) Characterization of two new glycosyl hydrolases from the lactic acid bacterium Carnobacterium piscicola strain BA. Appl Environ Microbol 67: 5094–5099.
[30] Cowan DA, Casanueva A, Stafford W (2007) Ecology and biodiversity of cold-adapted microorganisms. In: Physiology and biochemistry of extremophiles. American Society of Microbiology, Washington, DC.
[31] Damare S, Raghukumar C, Muraleedharan UD *et al* (2006) Deep-sea fungi as a source of alkaline and cold-tolerant proteases. Enzyme Microb Technol 39:172–181. https ://doi.org/10.1016/j.enzmi ctec.2006.03.032.
[32] De Gobba C, Tompa G, Otte J (2014) Bioactive peptides from caseins released by cold active proteolytic enzymes from Arsukibacterium ikkense. Food Chem 165:205–215. https ://doi.org/10.1016/j.foodc hem.2014.05.082.
[33] Dhiman SS, Garg G, Sharma J, Kalia VC, Kang YC, Lee JK (2014) Reduction in acute ecotoxicity of paper mill effluent by sequential application of xylanase and laccase. PLoS ONE 9:1–13.
[34] Dhiman SS, Sharma J, Bindu B (2008) Industrial applications and future prospects of microbial xylanases: a review. BioResources 3:1377–1402.
[35] Di Lauro B, Strazzulli A, Perugino G, La Cara F, Bedini E, Corsaro MM, Rossi M, Moracci M (2008) Isolation and characterization of a new family 42 β-galactosidase from the thermoacidophilic bacterium Alicyclobacillus acidocaldarius : identifi cation of the active site residues. Biochim Biophys Acta 1784:292–301.
[36] Dornez E, Verjans P, Arnaut F, Delcour JA, Courtin CM (2011) Use of psychrophilic xylanases provides insight into the xylanase functionality in bread making. J Agric Food Chem 59:9553–9562.
[37] Duarte AW, Dayo-Owoyemi I, Nobre FS, Pagnocca FC, Chaud LC, Pessoa A, Felipe MG, Sette LD (2013) Taxonomic assessment and enzymes production by yeasts isolated from marine and terrestrial Antarctic samples. Extremophiles 17:1023–1035.
[38] Farrell, J, Rose, A (1967) Temperature effects on microorganisms. *Annual Reviews in Microbiology, 21*(1), 101-120.
[39] Feller G, Gerday C (2003) Psychrophilic enzymes: hot topics in cold adaptation. Nat Rev Microbiol 1:200–208. https ://doi.org/10.1038/ nrmic ro773.
[40] Feller G, Le Bussy O, Gerday C (1998) Expression of psychrophilic genes in mesophilic hosts: assessment of the folding state of a recombinant α-amylase. Appl Environ Microbiol 64:1163–1165.
[41] Feller G, Lonhienne T, Deroanne C, Libioulle C, Van Beeumen J, Gerday C (1992) Purification, characterization, and nucleotide sequence of the thermolabile alpha-amylase from the Antarctic psychrotroph Alteromonas haloplanctis A23. J Biol Chem 267:5217–5221.
[42] Feller G, Gerday C (2003) Psychrophilic enzymes: hot topics in cold adaptation. *Nature reviews microbiology, 1*(3), 200.
[43] Ferrer M, Golyshina O, Beloqui A, Golyshin PN (2007) Mining enzymes from extreme environments. Curr Opin Microbiol 10:207–214.
[44] Festersen RM, Olsen HS, Pedersen S (2005) Alcohol product processes. Granted Patents DE04718914T1, EP1604019B1, CN1788083B, US8772001B2.
[45] Fickoblean E, Stuart CP, Boraston AB (2011) Structural analysis of CPF_2247, a novel α-amylase from Clostridium perfringens. Proteins 79:2771–2777
[46] Fornbacke M, Clarsund M (2013) Cold-adapted proteases as an emerging class of therapeutics. Infect Dis Ther 2(1):15–26. doi:10.1007/s40121-013-0002-x
[47] Fretwell, P., Pritchard, H. D., Vaughan, D. G., Bamber, J., Barrand, N., Bell, R., . . . Casassa, G. (2013) Bedmap2: improved ice bed, surface and thickness datasets for Antarctica.
[48] Georlette D, Blaise V, Collins T *et al* (2004) Some like it cold: biocatalysis at low temperatures. FEMS Microbiol Rev 28:25–42
[49] Gerday C, Aittaleb M, Bentahir M *et al* (2000) Cold-adapted enzymes: from fundamentals to biotechnology. Trends Biotechnol 18: 103–107. https://doi.org/10.1016/S0167-7799(99)01413-4.
[50] Gilbert JA, Dupont CL (2011) Microbial metagenomics: beyond the genome. Ann Rev Mar Sci 3:347–371.
[51] Giovannoni S, Stingl U (2007) The importance of culturing bacterioplankton in the 'omics' age. Nat Rev Microbiol 5:820–826.
[52] Gohel V, Duan G (2012) No-cook process for ethanol production using indian broken rice and pearl millet. Int J Microbiol 2012:680232. doi:10.1155/2012/680232
[53] Gupta R, Gigras P, Mohapatra H, Goswami VK, Chauhan B (2003) Microbial α-amylases: biotechnological perspective. Process Biochem 38:1599–1616.
[54] Gutshall K, Trimbur D, Kasmir J (1995) analysis of a novel gene and beta-galactosidase isozyme from a psychrotrophic Arthrobacter isolate. J Bacteriol 177:1981–1988.
[55] Gutshall K, Wang K, Brenchley JE (1997) A novel Arthrobacter β-galactosidase with homology to eucaryotic β-galactosidases. J Bacteriol 179:3064-3067.
[56] Handelsman J, Rondon MR, Brady SF, Clardy J, Goodman RM (1998) Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products. Chem Biol 5:R245- R249.
[57] Harris AD, Ramalingam C (2010) Xylanases and its application in food industry: a review. J Exp Sci 1:1-11.
[58] Hashim NHF, Bharudin I, Nguong DLS *et al* (2013) Characterization of Afp1, an antifreeze protein from the psychrophilic yeast Glaciozyma antarctica PI12. Extremophiles 17:63–73. https ://doi. org/10.1007/s0079 2-012-0494-4.
[59] Hausmann S, Jaeger K-E (2010) Lipolytic enzymes from bacteria. In: Timmis KN (ed) Handbook of hydrocarbon and lipid microbiology v. 2 (part 4). Springer, Berlin, pp 1099–1126.
[60] Heath C, Hu XP, Cary SC, Cowan D (2009) Identification of a novel alkaliphilic esterase active at low temperatures by screening a metagenomic library from antarctic desert soil. Appl Environ Microbiol 75:4657–4659
[61] Helmke E, Weyland H (2004) Psychrophilic versus psychrotolerant bacteria-occurrence and significance in polar and temperate marine habitats. Cell Mol Biol 50:553–561
[62] Hess M, Sczyrba A, Egan R, KimTW, Chokhawala H, Schroth G, *et al.* (2011) Metagenomic discovery of biomass-degrading genes and genomes from cow rumen. Science 331:463–467
[63] Horikoshi K (1999) Alkaliphiles: some applications of their products for biotechnology. Microbiol Mol Biol Rev 63:735–750
[64] Huston AL (2008) Biotechnological aspects of cold-adapted enzymes. In: Margesin R, Schinner F, Marx JC, Gerday C (eds) Psychrophiles: from biodiversity to biotechnology. Springer, Heidelberg, pp 347–363.
[65] Ji L, Yang J, Fan H, Yang Y, Li B, Yu X, Zhu N, Yuan H (2014) Synergy of crude enzyme cocktail from cold-adapted Cladosporium

cladosporioides Ch2-2 with commercial xylanase achieving high sugars yield at low cost. Biotechnol Biofuels 7(1):130. doi:10.1186/s13068-014-0130-x
[66] Karan R, Capes MD, Dassarma S (2012) Function and biotechnology of extremophilic enzymes in low water activity. Aquat Biol 8(1):4. doi:10.1186/2046-9063-8-4
[67] Karasová-Lipovová P, Strnad H, Spiwok V, Malá S, Králová B, Russell NJ (2003) The cloning, purifi cation and characterisation of a cold-active b-galactosidase from the psychrotolerant Antarctic bacterium Arthrobacter sp. C2-2. Enzyme Microb Technol 33:836–844
[68] Kim SM, Park H, Choi JI (2017) Cloning and characterization of cold-adapted α-amylase from Antarctic Arthrobacter agilis. Appl Biochem Biotechnol 181:1048
[69] Kirk O, Christensen MW (2002) Lipases from Candida antarctica: unique biocatalysts from a unique origin. Org Process Res Dev 6(4):446–451. doi:10.1021/op0200165
[70] Knietsch A, Waschkowitz T, Bowien S, Henne A, Daniel R (2003) Construction and screening of metagenomiclibraries derived from enrichment cultures: generation of a gene bank for genes conferring alcohol oxidoreductase activity on Escherichia coli. Appl Environ Microbiol 69:1408–1416
[71] Kosugi A, Murashima K, Doi RH (2002) Characterization of two noncellulosomal subunits, ArfA and BgaA, from Clostridium cellulovorans that cooperate with the cellulosome in plant cell wall degradation. J Bacteriol 184:6859–6865
[72] Kourist R, Hari Krishna S, Patel JS, Bartnek F, Hitchman TS, Weiner DP, Bornscheuer UT (2007) Identification of a metagenome-derived esterase with high enantioselectivity in the kinetic resolution of arylaliphatic tertiary alcohols. Org Biomol Chem 5:3310–3313
[73] Kuddus M, Ramteke PW (2012a) Recent developments in production and biotechnological applications of cold active microbial proteases. Crit Rev Microbiol 38:330–338. https ://doi. org/10.1155/2013/74285 9
[74] Kuddus M, Roohi Saima, Ahmad IZ (2012b) Cold-active extracellular α-amylase production from novel bacteria Microbacterium foliorum, GA2 and Bacillus cereus, GA6 isolated from Gangotri glacier, Western Himalaya. J Genet Eng Biotech 10:151–159
[75] Kumar A, Pillay B, Olaniran AO (2014) Two structurally different dienelactone hydrolases (TfdeI and TfdeII) from Cupriavidus necator JMP134 plasmid pJP4 catalyse cis- and trans-dienelactones with similar efficiency. PLoS ONE 9:e101801. https ://doi. org/10.1371/journ al.pone.01018 01
[76] Lambo A, Patel T (2006) Cometabolic degradation of polychlorinated biphenyls at low temperature by psychrotolerant bacterium Hydrogenophaga sp. IA3-A. Curr Microbiol 53(1):48–52
[77] Lario LD, Chaud L, Almeida M, Converti A, Sette LD, Pessoa A Jr (2015) Production, purification, and characterization of an extracellular acid protease from the marine Antarctic yeast Rhodotorula mucilaginosa L7. Fungal Biol 119:1129–1136
[78] Lee JH, Kim YS, Yeom SJ, Oh DK (2011) Characterization of a glycoside hydrolase family 42 β-galactosidase from Deinococcus geothermalis . Biotechnol Lett 33:577–583
[79] Liu, X.J., Zhang, J., Hui, H.P., Li, Z.J., (2016) Microbial production and characterization of poly-3-hydroxybutyrate by Neptunomonas Antarctica. PeerJ 4, e2291.
[80] Lorenz P, Eck J (2005) Metagenomics and industrial applications. Nat Rev Microbiol 3:510–516 Lorenz P, Liebeton K, Niehaus F, Eck J (2002) Screening for novel enzymes for biocatalytic processes: accessing the metagenome as a resource of novel functional sequence space. Curr Opin Biotechnol 13:572–577
[81] Lu M, Wang S, Fang Y, Li H, Liu S, Liu H (2010) Cloning, expression, purification, and characterization of cold-adaptedα-amylase from Pseudoalteromonas arctica GS230. Protein J 29:591–597
[82] Mahitha, G., Madhuri, J., (2016) Microbial polyhydroxybutyrate production by using cheap raw materials as substrates. Indian J. Pharm. Biol. Res. 4, 57–62.
[83] Margesin R, Feller G, Gerday C *et al* (2002) Cold-adapted microorganisms: adaptation strategies and biotechnological potential. In: Bitton G (ed) The encyclopedia of environmental microbiology, vol 2. Wiley, New York, pp 871–885
[84] Marx J-C, Collins T, D'Amico S *et al* (2007) Cold-adapted enzymes from marine Antarctic microorganisms. Mar Biotechnol 9:293–304. https ://doi.org/10.1007/s1012 6-006-6103-8
[85] Moiseeva OV, Solyanikova IP, Kaschabek SR *et al* (2002) A new modified ortho cleavage pathway of 3-chlorocatechol degradation by Rhodococcus opacus 1CP: genetic and biochemical evidence. J Bacteriol 184:5282–5292. https ://doi.org/10.1128/ JB.184.19.5282-5292.2002
[86] Morar M, Pengelly K, Koteva K, Wright GD (2012) Mechanism and diversity of the erythromycin esterase family of enzymes. Biochemistry 51:1740–1751
[87] Moreira LR, Filho EX (2016) Insights into the mechanism of enzymatic hydrolysis of xylan. Appl Microbiol Biotechnol 100:5205–5214
[88] Morita T (1966). T. Morita and T. Tanaka, Phys. Rev. 145, 288 (1966). *Phys. Rev., 145*, 288.
[89] Mykytczuk NC, Foote SJ, Omelon CR *et al* (2013) Bacterial growth at -15˚C; molecular insights from the permafrost bacterium Planococcus halocryophilus Or1. ISME J 7:1211–1226
[90] Nakagawa T, Ikehata R, Uchino M, Miyaji T, Takano K, Tomizuka N (2006) Cold-active acid β-galactosidase activity of isolated psychrophilic-basidiomycetous yeast Guehomyces pullulans . Microbiol Res 161:75–79
[91] Nardini M, Dijkstra BW (1999) α/β hydrolase fold enzymes: the family keeps growing. Curr Opin Struct Biol 9:732–737. https ://doi. org/10.1016/S0959 -440X(99)00037 -8
[92] Ogrydziak DM (1993) Yeast extracellular proteases. Crit Rev Biotechnol 13:1–55
[93] Oh KH, Seong CS, Lee SW *et al* (1999) Isolation of a psychrotrophic Azospirillum sp. and characterization of its extracellular protease. FEMS Microbiol Lett 174:173–178. https ://doi.org/10.1016/ S0378 -1097(99)00131 -7
[94] Pathak D, Ollis D (1990) Refined structure of dienelactone hydrolase at 1.8 Å. J Mol Biol 214:497–525. https ://doi.org/10.1016/0022- 2836(90)90196 –S
[95] Pawlak-Szukalska A, Wanarska M, Popinigis AT, Kur J (2014) A novel cold-active β-d-galactosidase with transglycosylation activity from the Antarctic Arthrobacter sp. 32cB—Gene cloning, purification and characterization. Process Biochem 49(12):2122–2133. doi:10.1016/ j.procbio.2014.09.018
[96] Pazgier M, Turkiewicz M, Kalinowska H, Bielecki S (2003) The unique cold-adapted extracellular subtilase from psychrophilic yeast Leucosporidium antarcticum. J Mol Catal B Enzym 21:39–42
[97] Poltorak OM, Chukhrai ES, Pilipenko OS, Atyaksheva LF, Beregalov AE (2007) A comparative study of the structure and properties of β-galactosidases. Russ J Phys Chem A 81:808–812
[98] Priscu JC, Christner BC (2004) Earth's icy biosphere *Microbial diversity and bioprospecting* (pp. 130-145): American Society of Microbiology.
[99] Pulicherla KK, Mrinmoy G, Kumar S *et al* (2011) Psychrozymes – the next generation industrial enzymes. J Mar Sci Res Dev 1:102.
[100] Qin Y, Huang Z, Liu Z (2014) A novel cold-active and salt-tolerant α-amylase from marine bacterium Zunongwangia profunda : mo-

-lecular cloning, heterologous expression and biochemical characterization. Extremophiles 18:271.
[101] Ram RJ, Verberkmoes NC, Thelen MP, Tyson GW, Baker BJ, Blake RC 2nd, Shah M, Hettich RL, Banfield JF (2005) Community proteomics of a natural microbial biofilm. Science 308:1915–1920.
[102] Ramli ANM, Mahadi NM, Shamsir MS *et al* (2012) Structural prediction of a novel chitinase from the psychrophilic Glaciozyma antarctica PI12 and an analysis of its structural properties and function. J Comput Aided Mol Des 26:947–961. https ://doi. org/10.1007/s1082 2-012-9585-7.
[103] Ray MK, Devi KU, Kumar GS, Shivaji S (1992) Extracellular protease from the Antarctic yeast Candida humicola. Appl Environ Microbiol 58:1918–1923.
[104] Robertson DE, Chaplin JA, DeSantis G, Podar M, Madden M, Chi E, *et al.* (2004) Exploring nitrilase sequence space for enantioselective catalysis. Appl Environ Microbiol 70: 2429–2436.
[105] Rosano GL, Ceccarelli EA (2009) Rare codon content affects the solubility of recombinant proteins in a codon biasadjusted Escherichia coli strain. Microb Cell Fact 8:41.
[106] Ruberto L, Vazquez S, Lobalbo A *et al* (2005) Psychrotolerant hydrocarbon-degrading Rhodococcus strains isolated from polluted Antarctic soils. Antarct Sci 17(1):47–56.
[107] Saba I, Qazi PH, Rather SA *et al* (2012) Purification and characterization of a cold active alkaline protease from Stenotrophomonas sp., isolated from Kashmir, India. World J Microbiol Biotechnol 28:1071–1079. https ://doi.org/10.1007/s1127 4-011-0905-1.
[108] Sabri, M., Melara, R. D., Algom, D. (2001). A confluence of contexts: Asymmetric versus global failures of selective attention to Stroop dimensions. *Journal of experimental psychology: Human perception and performance, 27*(3), 515.
[109] Saishin N, Ueta M, Wada A, Yamamoto I (2010) Properties of β-galactosidase purifi ed from Bifi dobacterium longum subsp. longum JCM 7052 grown on gum arabic. Int J Biol Macromol 10:23–31
[110] Santiago M, Ramírez-Sarmiento CA, Zamora RA, Parra LP (2016) Discovery, molecular Mechanisms, and industrial applications of cold-active enzymes. Front Microbiol 7:1408. https ://doi. org/10.3389/fmicb .2016.01408
[111] Sarmiento F, Peralta R, Blamey JM (2015) Cold and hot extremozymes: industrial relevance and current trends. Front Bioeng Biotechnol 3:148. doi:10.3389/fbioe.2015.00148
[112] Satoh T, Hosokawa M (2006) Structure, function and regulation of carboxylesterases. Chem Biol Interact 162:195–211
[113] Schlömann M (1994) Evolution of chlorocatechol catabolic pathways. Conclusions to be drawn from comparisons of lactone hydrolases. Biodegradation 5:301–321. https ://doi.org/10.1007/BF006 96467
[114] Schmeisser C, Steele H, StreitWR (2007) Metagenomics, biotechnology with non-culturable microbes. Appl Microbiol Biotechnol 75:955– 962
[115] Schmidt M, Stougaard P (2010) Identifi cation, cloning an expression of a cold-active β-galactosidase from a novel arctic bacterium, Alkalilactibacillus ikkense . Environ Technol 3:1107–1114
[116] Sediment Contam 14(3):281–291
[117] Shah, M.P., Patel, K.A., (2014) Microbial decolorization and degradation of remazol Black & Mordant Orange by microbial consortia isolated from common effluent treatment plant. Int. J. Environ. Bioremed. Biodegrad. 2, 117–124.
[118] Sheridan PP, Brenchley JE (2000) Characterization of a salt-tolerant family 42 β-galactosidase from a psychrophilic antarctic Planococcus isolate. Appl Environ Microbiol 66:2438–2444
[119] Siddiqui KS, Williams TJ, Wilkins D *et al* (2013) Psychrophiles. Annu Rev Earth Planet Sci 41:87–115. https ://doi.org/10.1146/annur ev-earth -04061 0-13351 4
[120] Simon C, Daniel R (2011) Metagenomic analyses: past and future trends. Appl Environ Microbiol 77:1153–1161
[121] Song C, Liu G-L, Xu J-L, Chi Z-M (2010) Purifi cation and characterization of extracellular β-galactosidase from the psychrotolerant yeast Guehomyces pullulans 17–1 isolated from sea sediment in Antarctica. Process Biochem 45:954–960
[122] Steele HL, Jaeger KE, Daniel R, StreitWR (2009) Advances in recovery of novel biocatalysts from metagenomes. J Mol Microbiol Biotechnol 16:25–37
[123] Stokes, DE (1963). Spatial models of party competition. *American political science review, 57*(2), 368-377.
[124] Suen W-C, Zhang N, Xiao L, Madison V, Zaks A (2004) Improved activity and thermostability of Candida antarctica lipase B by DNA family shuffling. Protein Eng Des Sel 17(2):133–140. doi:10.1093/protein/gzh017
[125] Thomas DN, Dieckmann GS (2002) Antarctic sea ice-a habitat for extremophiles. Science 295:641–644. https://doi.org/10.1126/science.1063391.
[126] Tobe S, Takami T, Ikeda S, Mitsugi K (1976) Production and some enzymatic properties of alkaline proteinase by Candida lipolytica. Agric Biol Chem 40:1087–1092
[127] Trimbur D, Gutshall K, Prema P, Brenchley JE (1994) Characterization of a psychrotrophic Arthrobacter gene and its cold-active-galactosidase. Appl Environ Microbiol 60:4544–4552
[128] Turkiewicz M, Pazgier M, Donachie SP, Kalinowska H (2005) Invertase and α-glucosidase production by the endemic Antarctic marine yeast Leucosporidium antarcticum. Polish Polar Res 26:125–136
[129] Turkiewicz M, Pazgier M, Kalinowska H, Bielecki S (2003) A cold-adapted extracellular serine proteinase of the yeast Leucosporidium antarcticum. Extremophiles 7:435–442
[130] Tyson GW, Chapman J, Hugenholtz P, Allen EE, Ram RJ, Richardson PM, Solovyev VV, Rubin EM, Rokhsar DS, Banfield JF (2004) Community structure and metabolism through reconstruction of microbial genomes from the environment. Nature 428:37–43
[131] Uchiyama T, Miyazaki K (2009) Functional metagenomics for enzyme discovery: challenges to efficient screening. Curr Opin Biotechnol 20:616–622
[132] Venter JC, Remington K, Heidelberg JF, Halpern AL, Rusch D, Eisen JA, *et al.* (2004) Environmental genome shotgun sequencing of the Sargasso Sea. Science 304:66–74
[133] Vieites JM, Guazzaroni ME, Beloqui A, Golyshin PN, Ferrer M (2009) Metagenomics approaches in systems microbiology. FEMS Microbiol Rev 33:236–255
[134] Wagner UG, Petersen EI, Schwab H, Kratky C (2002) EstB from Burkholderia gladioli: a novel esterase with a beta-lactamase fold reveals steric factors to discriminate between esterolytic and betalactam cleaving activity. Protein Sci 11:467–478
[135] Walia A, Guleria S, Mehta P, Chauhan A, Parkash J (2017) Microbial xylanases and their industrial application in pulp and paper biobleaching: a review. 3 Biotech 7:11. https ://doi.org/10.1007/ s1320 5-016-0584-6
[136] Wang E, Koutsioulis D, Leiros HKS *et al* (2007) Crystal structure of alkaline phosphatase from the Antarctic Bacterium TAB 5. J Mol Biol 366:1318–1331. https ://doi.org/10.1016/j.jmb.2006.11.079
[137] Wang G, Luo H, Wang Y *et al* (2011) A novel cold-active xylanase gene from the environmental DNA of goat rumen contents: direct cloning, expression and enzyme characterization. Bioresour Technol 102:3330–3336. https ://doi.org/10.1016/j.biort ech.2010.11.004
[138] Wang G, Wang Q, Lin X *et al* (2016) A novel cold-adapted and highly salt-tolerant esterase from Alkalibacterium sp. SL3 from the sediment of a soda lake. Sci Rep 6:19494. https ://doi.org/10.1038/ srep1 9494
[139] Wang K, Li G, Qin Y, Znang CT, Liu YH (2010) A novel metagenome-derived β-galactosidase: gene cloning, overexpression, purifi

cation and characterization. Appl Microbiol Biotechnol 88:155–165
[140] Welander U (2005) Microbial degradation of organic pollutants in soil in a cold climate. Soil
[141] Wen J, Ren C, Huang N, Liu Y, Zeng R (2015) Draft genome of bagasse-degrading bacteria Bacillus aryabhattai GZ03 from deep sea water. Mar Genomics 19:13–14. doi:10.1016/j. margen.2014.11.004
[142] White PL, Wynn-Williams DD, Russell NJ (2000) Diversity of thermal responses of lipid composition in the membranes of the dominant culturable members of an Antarctic fellfi eld soil bacterial community. Antarct Sci 72:386–393
[143] Xu K, Tang X, Gai Y, Mehmood M, Xiao X, Wang F (2011) Molecular characterization of coldinducible beta-galactosidase from Arthrobacter sp. ON14 isolated from Antarctica. J Microbiol Biotechnol 21:236–242
[144] Yooseph S, Sutton G, Rusch DB, Halpern AL, Williamson SJ, Remington K, *et al.* (2007) The Sorcerer II Global Ocean Sampling expedition: expanding the universe of protein families. PLoS Biol 5:e16
[145] Zaliha RN, Salleh AB, Basri M, Ali MSBM (2012) Cold active enzyme and method thereof. US Patent 2012/0058514
[146] Zhang DC, Yu Y, Xin YH *et al* (2008) Colwellia polaris sp. nov., a psychrotolerant bacterium isolated from Arctic sea ice. Int J Syst Evol Microbiol 58:1931–1934. https ://doi.org/10.1099/ijs.0.65596 -0
[147] Zhang H, Mu H, Mo Q *et al* (2016) Gene cloning, expression and characterization of a novel cold-adapted protease from Planococcus sp. J Mol Catal B Enzym 130:1–8. https ://doi.org/10.1016/j. molca tb.2016.04.002
[148] Zhang JW, Zeng RY (2008) Purification and characterization of a coldadapted α-amylase produced by Nocardiopsis, sp. 7326 isolated from Prydz Bay, Antarctic. Mar Biotechnol 10:75–82
[149] Zhang SC, Sun M, Li T *et al* (2011) Structure analysis of a new psychrophilic marine protease. PLoS One 6:e26939. https ://doi. org/10.1371/journ al.pone.00269 39
[150] Zheng X, Chu X, Zhang W *et al* (2011) A novel cold-adapted lipase from Acinetobacter sp. XMZ-26: gene cloning and characterisation. Appl Microbiol Biotechnol 90:971–980. https ://doi. org/10.1007/s0025 3-011-3154-1

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